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The T-381C SNP in *BNP* gene may be modestly associated with type 2 diabetes: an updated meta-analysis in 49 279 subjects

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A recent study reported an association between the brain natriuretic peptide (*BNP*) promoter T-381C polymorphism (rs198389) and protection against type 2 diabetes (T2D). As replication in several studies is mandatory to confirm genetic results, we analyzed the T-381C polymorphism in seven independent case–control cohorts and in 291 T2D-enriched pedigrees totalling 39 557 subjects of European origin. A meta-analysis of the seven case–control studies ($n = 39\,040$) showed a nominal protective effect [odds ratio (OR) = 0.86 (0.79–0.94), $P = 0.0006$] of the CC genotype on T2D risk, consistent with the previous study. By combining all available data ($n = 49\,279$), we further confirmed a modest contribution of the *BNP* T-381C polymorphism for protection against T2D [OR = 0.86 (0.80–0.92), $P = 1.4 \times 10^{-5}$]. Potential confounders such as gender, age, obesity status or family history were tested in 4335 T2D and 4179 normoglycemic subjects and they

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had no influence on T2D risk. This study provides further evidence of a modest contribution of the *BNP* T-381C polymorphism in protection against T2D and illustrates the difficulty of unambiguously proving modest-sized associations even with large sample sizes.

INTRODUCTION

The worldwide prevalence of type 2 diabetes (T2D) is projected to nearly double by the year 2025 (1). T2D is associated with an increased risk for nephropathy, end-stage renal disease, retinopathy, neuropathy, extremity amputations, cardiovascular disease and an overall increase in mortality risk (2).

T2D results from a complex interaction between genetic and environmental factors (3). So far, 17 genetic loci have been convincingly associated with T2D risk (4). Replication is a challenging but necessary step to validate new disease susceptibility genetic variants (5). Some of the most promising associations have indeed not been consistently replicated, suggesting that the original associations were spurious (5). Further, obesity status (6), family history of T2D (7), age (8), gender (9) and ethnicity (10) have been shown to modulate the effect of SNPs on T2D risk and they should be taken into consideration for an adequate interpretation of replication results.

Brain natriuretic peptide (BNP) is a member of the natriuretic family. In humans, BNP acts mainly as a cardiac hormone, produced primarily by the atrium and ventricle (11). BNP is synthetized in a pre-proBNP form in the ventricular myocardium, then cleaved into proBNP, itself equimolarly cleaved into the biologically active BNP and the inactive amino-terminal fragment (NT-proBNP) (12,13). The measurement of BNP and NT-proBNP concentrations are useful in establishing a diagnosis of heart failure. A variety of conditions can cause elevated natriuretic peptide levels: heart failure, acute coronary syndromes and renal dysfunction (14–16). Furthermore, the plasma levels of these two peptides are specifically influenced by age, gender and body mass index (BMI) (17,18). In the context of obesity, BNP levels are lower than expected, both in patients with and without heart failure (17,19). BNP is involved in lipolysis in human fat cells through a cyclic guanosine monophosphate (cGMP) signalling pathway (20,21).

Recently, starting from the hypothesis that *BNP* could be involved in metabolic diseases, Meirhaeghe *et al*. (22) studied the implication of the *BNP* T-381C (rs198389) polymorphism in T2D risk in 10 239 subjects and reported a protective role of the CC genotype (versus CT + TT) against T2D ($P = 0.008$). No study has confirmed this initial finding. This prompted us to assess the contribution of the T-381C variant to T2D in a large independent sample of European ancestry ($n = 39\,557$), using case–control and family-based studies. We then performed an updated meta-analysis of twelve independent case–control studies totalling 49 279 subjects.

RESULTS

Case–control studies

Clinical characteristics of the study populations are summarized in Table 1. We genotyped the *BNP* T-381C SNP in four case–control cohorts: a first set of 4157 T2D patients and 3628 controls of French origin, 178 T2D Swiss patients and 551 normoglycemic (NG) Swiss subjects from Zurich

Table 1. Clinical characteristics of the study groups

Study	Subjects	*n*	Sex ratio (men:women)	Age at examination (years)	Age at diagnosis (years)	BMI (kg/m^2)
Case–control studies	French T2D subjects	4157	2,380:1,777	60.84 ± 11.08	48.18 ± 10.47	31.47 ± 6.97
	French NG controls	3628	1,503:2,125	54.38 ± 9.60	—	25.52 ± 5.96
	Swiss 1 T2D subjects	178	78:100	50.06 ± 9.24	NA	46.2 ± 8.14
	Swiss 1 NG controls	551	112:439	49.27 ± 6.74	—	42.65 ± 7.29
	Swiss 2 T2D subjects	297	193:104	60.5 ± 0.5	50.0 ± 11.8	29.6 ± 5.9
	Swiss 2 NG controls	424	NA	NA	—	NA
	Austrian T2D subjects	426	254:172	56.67 ± 9.6	NA	30.44 ± 6.5
	Austrian NG controls	352	287:65	49.15 ± 5.84	—	26.2 ± 4.0
	DGI T2D cases[a]	1022	529:493	65.0 ± 10	58.0 ± 10	28.1 ± 4.1
	DGI NG controls[a]	1075	540:535	58.0 ± 10	—	27.6 ± 3.7
	FUSION T2D cases[a]	1161	653:508	63.4 ± 11.2	53.0 ± 12.0	29.8 ± 6.1
	FUSION NG controls[a]	1174	574:600	64.0 ± 11.7	—	26.8 ± 5.0
	DECODE T2D cases[a]	1405	829:576	68.4 ± 12.5	55.1 ± 12.4	30.1 ± 5.4
	DECODE NG controls[a]	23 190	7,313:15,877	59.7 ± 18.2	—	26.8 ± 5.0
Family-based study	T2D grand-parents	13	8:5	71.8 ± 9.0	54.9 ± 10.6	26.3 ± 3.8
	NG grand-parents	21	9:12	79.5 ± 8.9	—	26.9 ± 6.1
	T2D parents	91	42:49	61.4 ± 16.0	49.4 ± 15.4	26.4 ± 5.8
	NG parents	54	19:35	72.0 ± 9.7	—	25.9 ± 4.3
	T2D offspring	430	207:223	59.3 ± 9.9	47.6 ± 10.6	27.0 ± 4.6
	NG offspring	87	27:60	57.4 ± 10.0	—	25.1 ± 4.1

Data are mean ± SD. T2D, type 2 diabetes; NG, normoglycemic; DGI, Diabetes Genetics Initiative; FUSION, Finland-United States Investigation of NIDDM Genetics; NA, not available.
[a]Imputed genotypic data.

(set 1), all of them recruited for obesity surgery, an independently ascertained cohort from Switzerland, containing 297 Swiss T2D patients and 424 anonymous blood donors from CHUV of Lausanne (set 2), a cohort of 426 T2D and 352 Austria control subjects recruited in the area of Salzburg in Austrian. We also used imputed data from three studies. We used data from DIAGRAM, a recent meta-analysis of genome-wide association studies (23). Because a part of the Wellcome Trust Case Control Consortium (WTCCC) data was included in the original study, we only used data from Diabetes Genetics Initiative (DGI) (24), Finland-United States Investigation of NIDDM Genetics (FUSION) (25) and DECODE genetics (26,27) studies. We tested the impact of the *BNP* T-381C SNP on T2D in these seven independent case–control studies, but some of them were underpowered [statistical power of 15–93% to detect the effect (odds ratio, OR = 0.85) previously reported (22)]. Only one of these studies analyzed alone showed an association between the *BNP* T-381C polymorphism and risk for T2D: the DECODE study [OR = 0.77 (0.66–0.91), $P = 0.002$ under a recessive model]. The results from the seven case–control analyses are summarized in Table 2. This result was expected, since only two of the seven studies (4157 T2D and 3628 controls of French origin and 1405 T2D and 23 190 controls of Icelandic origin) achieved a statistical power >80% (93 and 81%, respectively). To increase statistical power, we therefore combined the seven case–control studies ($n = 39\,040$) in a meta-analysis and we observed a nominal protective effect [OR = 0.86 (0.79–0.94), $P = 0.0006$] of the CC genotype on T2D risk under a recessive model. This was consistent with a statistical power of 100% to observe the effect (OR = 0.85) originally described (22). By using dominant or additive models, we did not find more significant results than with the recessive model (data not shown). Neither genetic heterogeneity ($P = 0.71$) between studies nor publication bias ($P = 0.99$) was observed for this meta-analysis.

Heterogeneity with confounding variables

We then assessed the association of the *BNP* T-381C polymorphism with T2D with possible confounding variables such as obesity status, family history of T2D, age and gender (6–9). For these analyses, only the French and Swiss (set 1) subjects with documented data were used (4335 T2D and 4179 NG subjects). No heterogeneity was found between the French and Swiss (set 1) populations ($P = 0.20$), so we combined them for further analyses. Subgroup analyses did not show any association of these confounding variables with T2D ($0.31 < P < 0.99$, Materials and Methods and Supplementary Material, Table S1A). Case-only analyses of T2D patients after stratification for obesity status, family history of T2D, age of diagnosis and gender did not show any significant differences in the genotypic distribution ($P > 0.05$, Supplementary Material, Table S1B).

Family-based association studies

We also performed family-based association studies that are robust to population stratification (28,29). We genotyped the *BNP* T-381C SNP in 291 pedigrees selected for T2D (179 T2D subjects were overlapping with the first previously described French case–control design). Clinical characteristics of the family-based study are described in Table 1. We observed no distortion of transmission of the T risk allele in affected offspring (in 105 informative pedigrees, Z-score = −0.46, $P = 0.65$).

Table 2. Genotypic distributions and ORs for the *BNP* T-381C polymorphism and T2D among case–control studies

	n TT	TC	CC	OR [95% CI], *P*-value OR additive	OR recessive	OR dominant
French study						
Controls	1281	1734	613	—	—	—
Cases	1410	2059	688	0.98 [0.91–1.06], 0.71	0.93 [0.81–1.08], 0.35	1.01 [0.90–1.13], 0.85
Swiss 1 study						
Controls	184	266	101	—	—	—
Cases	65	87	26	0.87 [0.67–1.12], 0.28	0.75 [0.46–1.24], 0.27	0.87 [0.60–1.27], 0.48
Swiss 2 study						
Controls	154	205	65	—	—	—
Cases	112	142	43	0.95 [0.77–1.18], 0.67	0.93 [0.62–1.42], 0.75	0.94 [0.69–1.28], 0.7
Austrian study						
Controls	123	159	70	—	—	—
Cases	138	211	77	1.01 [0.83–1.24], 0.88	0.89 [0.62–1.27], 0.52	1.12 [0.83–1.51], 0.45
DGI study						
Controls	424	495	156	—	—	—
Cases	408	467	147	0.96 [0.83–1.12], 0.65	0.93 [0.67–1.29], 0.69	0.96 [0.76–1.19], 0.69
FUSION study						
Controls	468	531	175	—	—	—
Cases	482	534	145	0.92 [0.82–1.04], 0.10	0.82 [0.64–1.05], 0.11	0.94 [0.80–1.12], 0.51
DECODE study						
Controls	8520	10 962	3708	—	—	—
Cases	542	681	182	0.88 [0.81–0.97], 0.072	0.77 [0.66–0.91], 0.002	0.90 [0.79–1.02], 0.09

P-values adjusted for age, gender and BMI.

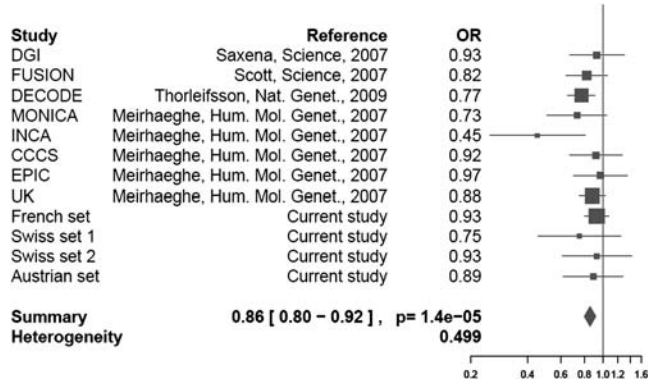

Figure 1. Meta-analysis of the association between *BNP* T-381C polymorphism and T2D risk. The meta-analysis includes 37 040 controls and 12 239 T2D cases. Summary illustrates the final OR under a recessive model; 95% CI is included in the pictogram.

Meta-analysis

Finally, we combined our current results (seven case–control studies, $n = 39\,040$) with the five case–control studies from Meirhaeghe *et al*. (22) in a meta-analysis of twelve studies ($n = 49\,279$). We detected a modest protective effect of the *BNP*-381CC genotype on T2D under a recessive model [OR = 0.86 (0.80–0.92), $P = 1.4 \times 10^{-5}$; Fig. 1]. Neither genetic heterogeneity ($P = 0.50$) between studies nor publication bias ($P = 0.31$) was observed for this overall meta-analysis.

DISCUSSION

Recently, Meirhaeghe *et al*. (22) proposed that the *BNP* T-381C SNP may protect against T2D in European populations. Our data, supported by cross-sectional case–control designs and a family-based study, showed a significant association of the *BNP* T-381C polymorphism with T2D in only one individual case–control study. However, by combining our seven case–control studies in a meta-analysis, we observed a modest protective effect [OR = 0.86 (0.79–0.94), $P = 0.0006$] against T2D. Using all the available published case–control data from twelve studies ($n = 49\,279$) in an overall meta-analysis, we confirmed further this effect with T2D [OR = 0.86 (0.80–0.92), $P = 1.4 \times 10^{-5}$].

Previous works in the field of T2D genetics (e.g. *PPARG* Pro12Ala, *KCNJ11* E23K and *WFS1* rs10010131) have shown the value of large scale meta-analyses to conclude about a true association, even if some individual studies were negative (30–32). Although our data provide additional confidence for a role for *BNP*, it remains below the conclusive thresholds of association usually proposed ($P < 10^{-7}$), despite a large sample size ($n = 49\,279$) (33). Nevertheless, the consistent direction of effect observed in the twelve case–control studies for this association between *BNP* rs198389 and T2D protection is striking (Fig. 1). Given this observation and the low level of between-study heterogeneity for the twelve study meta-analysis ($P = 0.50$), we are hopeful that future studies and updated larger meta-analyses will definitively confirm the association signal. Of note, it remains theoretically possible that a true association (especially in presence of between-study heterogeneity), may not be able to be replicated with consistency, no matter how large the studies (34).

Associations of genetic polymorphisms with T2D risk can be modulated by different confounding factors, such as family background, age, gender and obesity status (6–9). As recently reported for the K121Q polymorphism in *ENPP1* (35), the lack of replication for its association with T2D in individual studies may be due, in part, to heterogeneity between studies. Our data however exclude a major role of confounding variables for the *BNP* T-381C polymorphism and T2D risk.

To confirm a potential effect of the *BNP* T-381C functional variation (22) in T2D susceptibility, the physiological role of the BNP protein should be considered. *BNP* is a relevant candidate gene in energy metabolism and metabolic diseases, as it is a member of the natriuretic family involved in the regulation of blood pressure and blood volume (36), as well as in the control of lipolysis in human fat cells (20). As previously reported (22), the I-381C allele was associated with higher plasma BNP concentrations and higher BNP promoter activity in reporter gene assays, suggesting that relatively higher *BNP* expression may protect against T2D. However, the emerging genetic architecture of T2D strongly suggests that T2D susceptibility genes act predominantly through reduced beta-cell function (37,38) and marginally by insulin action (30,39) and up to now, no results have been published on a link between *BNP* and beta-cell function or insulin action. Further investigations are needed to understand the role of BNP in the complex molecular and physiological mechanisms involved in T2D.

In conclusion, the *BNP* T-381C SNP may have a modest effect in T2D-susceptibility. This study illustrates the difficulty of unambiguously proving association even with a large sample size ($n = 49\,279$). Although no universal threshold can be specified for statistical significance in all circumstances (28), our results clearly demonstrate the need for multiple-replication, large-scale meta-analysis studies and the use of stringent statistic thresholds in genetic association reports before concluding about a true association signal. Further investigation in additional populations of European ancestry is therefore of interest for the *BNP* T-381C SNP. A recent report showed a strong association between several gene variants at the *NPPA–NPPB/BNP* locus and plasma atrial natriuretic peptide, blood pressure or hypertension (especially the rs63279 in strong linkage disequilibrium ($r^2 = 0.87$ in HAPMAP CEU) with the T-381C SNP) (40). Beyond the genetic study of the *BNP* T-381C SNP, these data encourage us to investigate the full common SNP variation at the *NPPA–NPPB/BNP* in relation with glucose homeostasis and T2D risk.

MATERIALS AND METHODS

Subjects

The study protocol was approved by local Ethics Committees and an informed consent was obtained from each subject before participating in the studies. Glycaemic status was

defined according to 1997 American Diabetes Association criteria (41): NG, defined as fasting glucose < 6.1 mmol/l and T2D, defined as fasting plasma glucose ≥ 7.0 mmol/l and/or treatment by glucose lowering agents. All cases were either diagnosed with T2D or currently being treated for T2D. All control subjects were NG with an age at diagnosis 40 or more years.

Study I: original case–control design. The 4157 T2D cases and 3628 NG controls were French caucasians. They were recruited in different centres in France. For the T2D subjects: 1922 were patients from the Endocrinology–Diabetology Department at Corbeil-Essonnes Hospital, 726 were recruited by the CNRS UMR8090 Unit and 1509 came from the DIAB2.NEPHRO.GENE study (42). For the NG subjects: 1882 were participants of the D.E.S.I.R. prospective study (43), 853 were recruited by the CNRS-IPL and 893 came from the SUVIMAX (Supplementation en Vitamines et Minéraux Antioxidant) prospective cohort (44).

Study II: Swiss 1 replication cohort. We also genotyped 729 Swiss (178 cases and 551 controls), all of them were obese and recruited after gastric surgery in Zurich (45).

Study III: Swiss 2 replication cohort. To confirm the findings of studies I and II, we genotyped 297 additional Swiss T2D patients and 424 control subjects ascertained from anonymous healthy blood donors (CHUV of Lausanne).

Study IV: Austrian replication cohort. Four hundred and twenty-six Austrian individuals with T2D recruited from diabetes outpatient clinics of the Landeskliniken Salzburg and the Hallein Hospital and 352 Austrian control subjects from the Salzburg Atherosclerosclerosis Prevention Program in Subjects at High Individual Risk (SAPHIR) (46) were also genotyped.

Study V: DGI and FUSION replication cohorts. We included 1022 T2D cases and 1075 NG controls from the DGI stage 1 of Swedish and Finnish origin (24). The FUSION stage 1 sample had 1161 Finnish T2D cases and 1174 Finnish normal glucose-tolerant controls (25).

Study VI: DECODE genetics replication cohort. The recruitment of the 1405 T2D cases and the 23 190 controls from Icelandic origin has been described previously (26,27).

Study VII: T2D-selected pedigrees. In addition, we genotyped 291 French pedigrees recruited by UMR8090 for familial association tests. These families included 820 individuals, of whom 534 presented with T2D, 124 were glucose intolerant and 162 were NG subjects.

Genotyping

The *BNP* T-381C SNP was genotyped using the TaqMan technology (Applied Biosystems). Conditions for the TaqMan reaction were 95°C for 10 min and followed by 50 cycles of 95°C for 15 s, 60°C for 1 min.

Quality controls

The genotypic distribution of *BNP* T-381C SNP was in Hardy–Weinberg equilibrium ($P > 0.05$) for all case–control studies. There was a genotyping success rate of 98%, and a concordance rate of 100% when analyzing 384 duplicated DNA samples. Genotyping was also controlled in familial designs, using the PedCheck program (47). In pedigrees with T2D, we observed one incompatibility in 291 pedigrees.

Statistical analysis

The DeFinetti program was used to test the deviation from Hardy–Weinberg equilibrium (http://linkage.rockefeller.edu). The Woolf test was applied to assess the genotypic heterogeneity between Swiss and French populations. The statistical power was calculated with the QUANTO software, using the total number of cases and controls, the current prevalence of T2D in France (3.06% as reported by ALFEDIAM: http://www.alfediam.org), the OR resulting from the first meta-analysis (22) and an alpha-level *P*-value of 0.05. For the family-based association test, we used the FBAT program (Family Based Association Testing software). The association with T2D was estimated using a logistic regression model adjusted for age, gender and BMI. For the meta-analysis, fixed-effect summary estimates were calculated for a recessive model using the 'rmeta' and 'meta'packages of the R-Project (http://www.r-project.org). Subgroup analyses shown in Supplementary Material, Table S1A were done as followed: obese (BMI ≥ 30 kg/m^2) T2D patients versus obese NG controls, non-obese (BMI < 30 kg/m^2) T2D subjects versus non-obese NG subjects, T2D subjects with or without familial T2D history versus the whole NG control sample, T2D subjects with young onset T2D (≤ 48 years) or with late onset T2D (> 48 years) versus the whole NG control subset and finally T2D men versus NG men and T2D women versus NG women. We used the recessive model as previously proposed (22). However, the additive and dominant models are presented in Table 2. We also used Egger's regression method to test for publication bias (48). SPSS (version 14.0.2) and R (version 2.5.0) software were used for general statistical analyses.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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